# A STUDY OF THE CONCENTRATION OF THE ANTIBODIES IN THE BODY FLUIDS OF NORMAL AND IMMUNE ANIMALS.*

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THE presence of antibodies of various kinds in serum has long been known, and the concentration in that fluid has been carefully studied. The presence of antibodies in the various other body fluids has not been so carefully investigated, nor has sufficient allowance been made for individual variations in animals of the same species. While the authors were associated with Dr. Carlson in his work on lymph formation, he suggested that a careful comparison between the concentration of the antibodies in the various body fluids of the same animal might be of considerable importance in determining the differences between the lymph and serum, and in that way throw light on some of the problems of lymph formation, and possibly also on the point of origin of antibodies. When the work was begun, we intended to collect lymph from the different organs, but the practical difficulties encountered in introducing cannulae into the delicate lymphatics of such organs as the spleen was so great, that the project was temporarily abandoned, and the work has been confined to a comparison between serum, lymph from the cervical lymphatics, lymph from the thoracic duct, pericardial fluid, cerebrospinal fluid, and aqueous humor. Thus far work has been done on the hemolysins, hemagglutinins, agglutinins for the typhoid bacillus, the protein precipitins, and the opsonins, bacterial and erythrocytic. No work has yet been undertaken on the bacteriolysins. We have not enough data to enable us to draw any broad conclusions, and we will content ourselves with presenting what we believe to be the facts under the various conditions studied.

**Literature.**—The first studies that we were able to find on the relative concentration of antibodies in the various body fluids were those of Pegano,[36] who found the concentration of hemolysins of the thoracic lymph in dogs lower than that of the serum. Falloise [11] and later Batelli[4] confirmed the work of Pegano. Hughes and Carlson [8] working on normal dogs, horses, and cats found the concentration of hemolysins for

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rabbit corpuscles in the body fluids to form a descending series: serum, thoracic lymph, neck lymph, pericardial fluid, aqueous humor. No lysins were found in the cerebrospinal fluid. Straus and Wolf[40] studied the hemolytic power against rabbit corpuscles of the cerebrospinal fluid, edema fluid, pleural and pericardial transudates, and blister fluid, and attempted to correlate the hemolytic strength with the protein content. Marshall and Morgenroth[25] found anti-complement and anti-amboceptor in a pathological exudate—an ascites fluid. Hedinger [15] studied the hemolytic power of non-inflammatory exudates like those arising from cirrhosis of the liver and heart failure, and found that they were not so hemolytic as the serum. The inflammatory exudates arising from cases of tuberculosis and carcinoma were not so strongly hemolytic as non-inflammatory exudates. He failed to find hemolysins in the fluid from an ovarian cyst, or in the cerebrospinal fluid in two cases of tuberculosis. Marshall[24] found that pleural and ascites fluids were more strongly hemolytic than the serum from an infant. But no conclusions can be drawn from this comparison in regard to the comparative hemolytic power of serum and other body fluids in the same individual. He found a multiplicity of amboceptors and complements in the fluids that he studied. Grollo [4] could find no amboceptors for rabbit corpuscles in transudates, but found them in exudates, altho in the latter complement is often lacking. He suggests this method as a means of diagnosis between transudates and exudates. Lüdke[22] confirmed the findings of Marshall in regard to the hemolytic strength of transudates and exudates. Granström[13] found wide variations in the hemolytic content of transudates and exudates, and could establish no characteristics essential for either. The hemolysins did not run parallel with those of the blood. Isolysins are found less frequently in transudates and exudates than in the blood. Hemolysins were not found in the cerebrospinal fluid. Isolysins and heterolysins were found independent of the albumen content, number of the leukocytes, and the osmotic pressure of the fluids tested. Tedeschi[4] found precipitins in both transudates and exudates, less frequently in the latter than in the former. Mioni[30] found amboceptor but no complement for guinea-pig corpuscles in the pericardial fluid of the ox. Bard[2] claims to have found hemolysins in the cerebrospinal fluid of patients, and found that they were increased during various diseases. Massaglia[26] could not confirm the work of Bard. His results in both healthy and diseased individuals were negative. The presence of antibodies for syphilitic material in the cerebrospinal fluid has been shown by various investigators, among them Morgenroth and Stertz,[31] and Wassermann and Plaut.[42] Gatti [2] could demonstrate no hemolysins in the aqueous humor of the ox. Levaditi [2] showed that there is normally no opsonin in the aqueous humor; but if the fluid of the anterior chamber of the eye of an immune animal is withdrawn, the newly formed aqueous humor will contain opsonin. Böhme[6] investigated the opsonin content of pleural, peritoneal, and abscess fluids. He found that usually in such cases the opsonin content of the fluid was reduced for the infecting organism, but remained unchanged for other bacteria. He could find no opsonin in normal cerebrospinal fluid, but found them there after an inflammation had been set up in the dura. He could not develop opsonins in the cerebrospinal fluid by repeated puncture as Levaditi had done by drawing off the aqueous humor. He believes that there is a relation between the protein content and the opsonin action of a fluid.

**Methods.**—The plan of study adopted was to determine first the concentration of the antibodies in the body fluids of normal cats and dogs; then the concentration in actively immunized animals; and, finally, to study the passage of the antibodies from

the blood into the other body fluids in animals passively immunized by the withdrawal of large quantities of blood, and the injection of a corresponding amount of warm, defibrinated blood from an actively immunized animal.

The body fluids were secured under as nearly aseptic conditions as possible The animal was anesthetized with ether, and kept in a state of complete anesthesia, by the administration of the vapor through a trachea cannula or through a tube introduced through the larynx. The neck lymphatics were then isolated, and small, sterile, glass cannulae provided with sterile, rubber tubing were inserted. If there was no free flow of lymph, the neck was gently massaged. The lymph was never allowed to come in contact with the air of the room, for as soon as it filled the cannula and a part of the rubber tubing, it was drawn off by means of a fine, sterile Pasteur pipette, and placed in a dry, sterile test tube plugged as for bacteriological work. The lymph was allowed to coagulate spontaneously in the test tube, and was then defibrinated, and the delicate coagulum removed.

The thoracic duct was tied off at the same time as the isolation of the neck lymphatics so that the lymph formed during the experiment was retained in the duct. Usually the lymph from this duct was not collected until the animal had been bled to death, altho sometimes it was collected simultaneously with the neck lymph. The routine method was to draw the lymph by means of a Pasteur pipette provided with a bulb, so that the fluid never came in contact with the air at all. This fluid was also defibrinated.

The pericardial fluid was never collected until after the death of the animal by very complete bleeding from the arteries and veins of the neck. The thorax was opened by removing the sternum, a small hole was cut into the pericardium, and the fluid was removed by means of a sterile Pasteur pipette.

We found it a good plan in our experiments to suspend the animal by the jaws for a few minutes before attempting to withdraw the cerebrospinal fluid. This drained away the blood from the head and made admixture of this fluid with blood less likely. Our method was to open the dura between the first and the second cervical vertebrae, and then remove the fluid by means of the Pasteur pipette. The end of the pipette must be well rounded in the flame, otherwise rupture of the delicate blood vessels of the meninges is likely to follow.

The method of collecting the aqueous humor was simple and easy. It consisted in thrusting a sharp pointed Pasteur pipette into the anterior chamber of the eye through the cornea, and allowing the aqueous humor to flow into it, largely by the tension within the eyeball. A little suction sufficed to remove the last drop of the fluid.

The serum was secured from blood drawn when the animal was bled to death, and in most cases was freed from the corpuscles at once.

Careful notes were made in regard to the condition of the fluids, and in most cases where there was any admixture of blood, the fluid was discarded.

## HEMOLYSIS AND HEMAGGLUTININS.

**Methods.**—The study of the hemolysins and hemagglutinins in normal* animals was made on dogs only. The animals utilized were brought in from various parts of the city. Most of the tests were made with rabbit corpuscles in 5 per cent suspension in 0.9 per cent NaCl solution. In some cases rat and horse corpuscles were used.

* The term "normal" means animals which had not previously been immunized by us. We had no way of knowing what their history had been previous to coming to the laboratory.

Our methods were the following: Quantities of the various body fluids of the animal to be tested varying between 0.1 c.c. and 0.0001 c.c. were placed in a series of eight dry, sterile test tubes plugged as for bacteriological work. In order to make the necessary measurements with a pipette graded to $\frac{1}{100}$ of a c.c., dilutions of the body fluids $\frac{1}{10}$ and $\frac{1}{100}$ were made. To the fluid in each tube enough sterile 0.9 per cent NaCl solution was added to make the total volume up to 0.4 c.c. To this was then added 0.2 c.c. of a 5 per cent suspension of the corpuscles to be tested. In this way we got dilutions of the fluid varying between 1:6 and 1:6,144. All of the fluids from the same animal were prepared, the tubes were placed in a block containing a suitable number of holes, and adjusted to the sliding platform of a shaker in an incubator warmed to 37° C. The shaker was run by water power, and the motion was rapid enough to secure constant, thorough agitation, but not violent enough to injure the corpuscles. The routine technic was to keep the tubes in the shaker for an hour and in the ice-box from 12 to 20 hours to permit sedimentation of the corpuscles before the final reading.

In determining the amount of hemolysis in the final reading, the following method was employed: A measured sample of the corpuscle suspension in the test was sedimented in the centrifuge, and the supernatant liquid drawn off with a pipette. The corpuscles were then laked by adding distilled water to restore the original volume. This sample contained three times as much hemoglobin as the hemolytic tests, because 0.2 c.c. of the corpuscles were added to 0.4 c.c. of the fluid tested. Therefore, the above sample was diluted to three times its volume with water. This, then, would give exactly the same concentration of hemoglobin as in any tube in the test, provided that the hemolysis was complete, and is termed 100 per cent for this sample of corpuscles. By further dilution tubes containing 90 per cent, 80 per cent, etc., were prepared. No attempt was made to estimate closer than 10 per cent. A new scale was made for each sample of corpuscles.

The agglutinins were read from the same tubes as the hemolysins. The method employed to determine whether or not agglutination had occurred, was inspection of the rim of sedimented corpuscles. When the corpuscles, after sedimentation by standing in the ice-box, show a perfectly smooth, knife-edged border, no agglutination has occurred. If the border is slightly or decidedly roughened, agglutination has occurred At first this method was carefully supplemented by microscopic examination, but it was soon found so accurate that in the later experiments we depended entirely upon the observation of the rim of the corpuscles, and dispensed with the use of the microscope.

**A. Normal animals.**—The concentration of lysins and hemagglutinins in the body fluids of normal animals varies within rather narrow limits. This variation is great enough, however, to make it necessary that the comparison be made between the body fluids of the same animal. The following experiment shows the behavior of the body fluids of the normal dog.

Table I shows that the concentration of hemolysins is greater in the serum than in the other body fluids; thoracic lymph is next, at least in the case of rabbit corpuscles; and neck lymph is third.

This difference in the hemolytic power of serum or other body fluid against the corpuscles of different species of animals has been explained by Ehrlich and his coworkers on the basis of a multiplicity of amboceptors and complements, some of which are specific, some

TABLE 1.

COMPARATIVE HEMOLYTIC AND AGGLUTINATING POWER OF THE BODY FLUIDS OF A NORMAL DOG ON RABBIT AND RAT CORPUSCLES.

| DILUTION | SERUM | | | | NECK LYMPH | | | | THORACIC LYMPH | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rabbit | | Rat | | Rabbit | | Rat | | Rabbit | | Rat | |
| | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. |
| 1:6 | 100 | − | 10 | + | 5 | + | 0 | 0 | 40 | + | 0 | + |
| 1:12 | 40 | + | 0 | 0 | 0 | + | 0 | 0 | 0 | + | 0 | + |
| 1:24 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | + |
| 1:48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | sp | − |
| 1:96 | 0 | 0 | sp* | − | 0 | 0 | sp | − | 0 | 0 | 0 | 0 |
| 1:384 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| DILUTION | PERICARDIAL FLUID | | | | CEREBROSPINAL FLUID | | | | AQUEOUS HUMOR | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rabbit | | Rat | | Rabbit | | Rat | | Rabbit | | Rat | |
| | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. |
| 1:6 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | sp | − |
| 1:12 | 0 | + | 0 | 0 | 0 | 0 | sp | − | 0 | 0 | sp | − |
| 1:24 | 0 | 0 | sp | − | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1:48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | sp | − |
| 1:96 | 0 | 0 | 0 | 0 | 0 | 0 | sp | − | 0 | 0 | 0 | 0 |
| 1:384 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

*It will be noted that in this table several tubes are marked "sp." By that symbol is meant hemolysis not due to the ordinary hemolysins. The appearance of the partially laked corpuscles is entirely different from that in the ordinary hemolytic test. The hemoglobin can be seen diffusing from the sedimented corpuscles, while the supernatant fluid remains perfectly clear. The hemoglobin has the peculiar reddish purple tint of reduced hemoglobin, instead of the clear red of oxyhemoglobin. Furthermore, laking may appear anywhere in the series, frequently, where no hemolysis is to be expected, and is met more often in fluids like the cerebrospinal, or aqueous humor, which are normally not hemolytic, than in the other fluids. Rat corpuscles seem more susceptible to this form of hemolysis than rabbit corpuscles. Complement seems to inhibit this form of hemolysis.

non-specific. In most cases the thoracic lymph of normal dogs is hemolytic for rat corpuscles, altho in the experiment cited above, such was not the case.

As may be seen from the table above, the concentration of agglutinins may be higher in the thoracic lymph than in the serum. Such, however, is not the usual finding. In 10 experiments on normal dogs we found in seven the concentration of agglutinins highest in the serum; in two it was highest in the thoracic lymph; and in one

the concentration was the same in both. The fact that the concentration of agglutinins may be greater in the thoracic lymph than in the serum, renders it hard to see how these antibodies can come from the blood by pure filtration, for in that case, we should expect the hemolysins to run a parallel course—a thing which they do not do—or else we must assume that the agglutinins pass through membranes more readily than the hemolysins. It would be necessary, also, on the basis of filtration, to assume sudden great changes in the concentration of the agglutinins in the blood, for on no other basis could we explain the fact that the concentration of agglutinins would be so much lower in the serum by the time the lymph reached the upper end of the thoracic duct, than it was at the time the lymph was formed. Of course other explanations are possible: there may be an active secretion of the agglutinins into the lymph from the blood, or the agglutinins, after being formed in the area drained by the thoracic duct, are thrown into the lymph, reaching the blood by that route. Much more investigation must be made before any conclusion can be reached on this point.

The pericardial fluid when collected under the best conditions never shows hemolysins for rabbit corpuscles. Agglutinins may or may not be present. In four of our ten supposedly normal dogs hemolysis was noted, in only one case amounting to more than 10 per cent. Of these four animals, two were in poor condition, emaciated, and generally run down, and both these dogs yielded excessive amounts of pericardial fluid; in the other two cases, the pericardial was found to contain a few erythrocytes. Agglutinins were found in all four of these cases and in three others, making a total of seven in ten. From these experiments we are inclined to believe that hemolysins are not found in the pericardial fluid of normal dogs. The fact that some animals showed hemolysins in the pericardial fluid we would explain as a pericardial transudate in two cases, and to admixture with blood in two cases. We did not test whether it was amboceptor, or complement, or both which was absent from the fluid, altho we have evidence on this point in immune animals. Agglutinins for rabbit corpuscles may or may not be present in the pericardial fluid of normal dogs.

As will be seen from Table 1 the cerebrospinal fluid and aqueous

humor of normal animals contain no lysins or agglutinins for rat or rabbit corpuscles. In our 10 experiments on normal animals there were no traces of hemolysis or agglutination in a single case where admixture of blood was eliminated. Our results with cerebrospinal fluid confirm those of Massaglia, who could find no lysins in that fluid, and are contrary to those of Bard, who claims to have demonstrated them there.

**Conclusions.**—1. In the normal dog hemolysins for rabbit corpuscles are found in the serum, neck lymph, and thoracic lymph, but are absent from the pericardial fluid, cerebrospinal fluid, and aqueous humor. They are most concentrated in the serum, less concentrated in the thoracic lymph, and are found only in traces in the neck lymph.

2. Agglutinins are found in the serum, neck lymph, and thoracic lymph of normal dogs. They may or may not be present in the pericardial fluid, and are always absent from cerebrospinal fluid and aqueous humor. In most cases the concentration descends in the following order: serum, thoracic lymph, neck lymph, pericardial fluid; altho in some cases, the order is thoracic lymph, serum, neck lymph, pericardial fluid.

3. Serum and thoracic lymph show a weaker hemolysis and agglutination toward rat than toward rabbit corpuscles. Neck lymph lakes and agglutinates rabbit but not rat corpuscles. Pericardial fluid agglutinates rabbit but not rat corpuscles. Cerebrospinal fluid and aqueous humor neither lake nor agglutinate rat or rabbit corpuscles.

**B. Immunized animals.**—Various methods of producing active immunity were employed with good success. It is of interest to ascertain what methods of immunizing yield the best results. We employed the following: (1) Immunization of dogs with rabbit blood: (*a*) intraperitoneally by a single large injection of from 80 to 150 c.c. of blood, (*b*) intraperitoneally by repeated small injections, (*c*) subcutaneously by repeated small injections. (2) Immunization of dogs with horse serum:* (*a*) by a single large intraperitoneal injection of 100–150 c.c., (*b*) by repeated, small intraperitoneal injections, (*c*) by

* This serum was secured aseptically, November, 1908, by drawing the blood from the carotid of a horse into jars. It was allowed to coagulate and stand in the ice-box until the serum came out. The serum was then sealed into bulbs and kept in the ice-box until used.

repeated, small subcutaneous injections. A comparison of the results secured by using the fluids directly from the animal, so far as the lysins are concerned, is not conclusive, for, as will be seen, complement is not increased, at least not in proportion to the amboceptors, if at all. This fact necessitates the use of sufficient complement to supply all the amboceptors present to demonstrate the true state of affairs. So far as the agglutinins are concerned, apparently a single large dose of the serum or blood may develop them more markedly than the other methods tried. The repeated, small intraperitoneal injections yielded the most uniform results.

As has been noted by numerous investigators, the increase in complement does not keep pace with the increase in amboceptors. The apparent increase in the hemolysins and in the agglutinins for rabbit corpuscles is shown in Table 2.

TABLE 2.

LYTIC AND AGGLUTINATING ACTION OF THE BODY FLUIDS OF A DOG IMMUNIZED WITH RABBIT BLOOD. (November 25, December 15, 10 c.c. rabbit blood intraperitoneally; December 15, 15 c.c. Fluids collected December 23.)

| DILUTION | SERUM | | NECK LYMPH | | THORACIC LYMPH | | PERICARDIAL FLUID | | CEREBROSPINAL FLUID | | AQUEOUS HUMOR | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. |
| 1:1½ | — | — | — | — | — | — | o | + | o | o | o | o |
| 1:3 | — | — | — | — | — | — | o | + | o | o | o | o |
| 1:6 | 100 | + | 60 | + | 100 | + | o | + | o | o | o | o |
| 1:12 | 100 | + | o | + | 60 | + | o | + | o | o | o | o |
| 1:24 | 50 | + | o | + | o | + | .. | .. | .. | .. | .. | .. |
| 1:48 | o | + | o | + | o | + | .. | .. | .. | .. | .. | .. |
| 1:96 | o | + | o | + | o | + | .. | .. | .. | .. | .. | .. |
| 1:384 | o | + | o | + | o | + | .. | .. | .. | .. | .. | .. |
| 1:1,536 | o | + | o | o | o | o | .. | .. | .. | .. | .. | .. |
| 1:6,144 | o | o | o | o | o | o | .. | .. | .. | .. | .. | .. |

From Table 2 it can be seen by comparison with Table 1 that the repeated injections of rabbit blood does not appear to increase to any very marked extent the hemolytic power of the body fluids over that of a normal animal.

There is, however, a marked increase in the power of the body fluids to agglutinate rabbit corpuscles in those fluids which had the power to agglutinate them previous to the injection; there is no development, except in a few cases, of agglutinins in the cerebrospinal fluid and the aqueous humor. Altho Table 2 does not show this point, a careful comparison of this table with the succeeding ones

will show that the relative concentration of the agglutinins, in the body fluids, remain the same during the process of immunization.

That there is, however, a marked increase in the amboceptor content of the body fluids, normally containing them, during the process of immunization is shown by Table 3.

TABLE 3.

LYTIC AND AGGLUTINATING POWER ON RABBIT CORPUSCLES OF THE BODY FLUIDS OF DOG IMMUNIZED WITH RABBIT BLOOD AS AFFECTED BY COMPLEMENT (0.2 C.C. FRESH GUINEA-PIG SERUM).
(Intraperitoneal injections of rabbit blood as follows: December 1, 5 c.c.; December 5, 7½ c.c.; December 10, 10 c.c.; December 15, 15 c.c.; December 21, 16 c.c.; January 16, 20 c.c. Fluids collected February 8.)

| DILUTION | SERUM | | | | NECK LYMPH | | | | THORACIC LYMPH | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No Complement | | Complement | | No Complement | | Complement | | No Complement | | Complement | |
| | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. |
| 1:6 | 100 | − | 100 | − | 60 | + | 70 | + | 100 | − | 100 | − |
| 1:12 | 100 | − | 100 | − | 10 | + | 60 | + | 70 | − | 100 | − |
| 1:24 | 90 | + | 100 | − | 0 | + | 40 | + | 20 | + | 60 | + |
| 1:48 | 10 | + | 100 | − | 0 | + | 20 | + | 0 | + | 50 | + |
| 1:96 | 0 | + | 50 | + | 0 | 0 | 0 | 0 | 0 | + | 30 | + |
| 1:384 | 0 | + | 30 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1:1,536 | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1:6,144 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| DILUTION | PERICARDIAL FLUID | | | | CEREBROSPINAL FLUID | | | | AQUEOUS HUMOR | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No Complement | | Complement | | No Complement | | Complement | | No Complement | | Complement | |
| | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. |
| 1:6 | 0 | + | 20 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1:12 | 0 | + | 10 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1:24 | 0 | + | | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1:48 | 0 | + | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1:96 | 0 | 0 | 0 | 0 | .. | .. | .. | .. | .. | .. | .. | .. |
| 1:384 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 1:1,536 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 1:6,144 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |

This experiment shows very clearly that in the immunized animal the serum, neck lymph, thoracic lymph, and pericardial fluid do not contain complement in sufficient quantity to activate all of the amboceptor present in the fluid, because the addition of guinea-pig complement,* in doses of itself not lytic, is able to produce stronger

* We experienced considerable difficulty in securing a complement which was effective and at the same time did not of itself produce hemolysis. Rabbit serum, guinea-pig serum, and dog serum from which the amboceptor had been separated in the cold were tried. The guinea-pig serum proved the only effective one.

hemolysis where only traces had appeared, and to produce hemolysis in other cases where there were no traces with exactly the same amount of the fluid without complement.

The addition of complement in a non-hemolytic dose is able to cause hemolysis in the pericardial fluid. In 8 of 13 experiments on blood-immune dogs traces of hemolysis occurred in the lowest dilutions of that fluid. In these experiments we could detect no contamination with blood, neither did we note that the fluid was present in excessive amounts. Apparently, then, in dogs immune to a foreign blood, amboceptor is always, and complement usually present in the pericardial fluid. This agrees in part with the findings of Mioni who found amboceptor but no complement for guinea-pig corpuscles in the pericardial fluid of the ox. No amboceptors are found in the cerebrospinal fluid or aqueous humor, for no hemolysis occurs when effective complement is added, at least a complement which proved effective in the case of the other body fluids.

The agglutinins in the various body fluids are seen from Table 5 to run practically the same course as in the normal animal, except that the concentration is highest in the serum, a little lower in the thoracic lymph, still lower in the neck lymph, and lowest, but always present, in the pericardial fluid. Sometimes, as in the normal animal, the concentration of agglutinins in the thoracic lymph is equal to or greater than that of the serum. In the 16 immune animals in which we compared serum and thoracic lymph, in 11 cases the serum was higher in the concentration of the agglutinins than the thoracic lymph, in two cases the thoracic lymph was higher than the serum, and in three cases the two fluids showed equal concentration. The fact that the thoracic lymph may contain these antibodies in equal or even considerably higher concentration than the corresponding serum may be of significance as bearing upon the source of these substances.

In 15 experiments with cerebrospinal fluid from immune dogs, a positive agglutination was secured in two. In 16 experiments with the aqueous humor, positive results were secured in five. Thus it will be seen that agglutinins may be found in the cerebrospinal fluid and the aqueous humor, but their presence is the exception and not the rule.

A point of considerable interest is the determination whether the

fluids of an animal immune to one kind of blood show an increased hemolytic and agglutinating power toward the corpuscles of another species. An experiment of this kind is shown in Table 4.

TABLE 4.

LYTIC AND AGGLUTINATING ACTION ON HORSE AND RABBIT CORPUSCLES OF BODY FLUIDS OF A DOG INJECTED WITH HORSE SERUM.

(100 c.c. of serum injected intraperitoneally January 16; fluids removed January 29.)

| DILUTION | SERUM | | | | NECK LYMPH | | | | THORACIC LYMPH | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Horse Corpuscles | | Rabbit Corpuscles | | Horse Corpuscles | | Rabbit Corpuscles | | Horse Corpuscles | | Rabbit Corpuscles | |
| | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. |
| 1:6 | 100 | − | 100 | − | 100 | − | 50 | + | 100 | − | 100 | − |
| 1:12 | 100 | − | 100 | − | 90 | + | 10 | + | 100 | − | 40 | + |
| 1:24 | 100 | − | 30 | + | 60 | + | 0 | + | 50 | + | 0 | + |
| 1:48 | 70 | + | 10 | + | 10 | + | 0 | 0 | 20 | + | 0 | 0 |
| 1:96 | 30 | + | 0 | + | 0 | + | 0 | 0 | 0 | + | 0 | 0 |
| 1:384 | 0 | + | 0 | 0 | 0 | + | 0 | 0 | 0 | + | 0 | 0 |
| 1:1,536 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 |
| 1:6,144 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| DILUTION | PERICARDIAL FLUID | | | | CEREBROSPINAL FLUID | | | | AQUEOUS HUMOR | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Horse Corpuscles | | Rabbit Corpuscles | | Horse Corpuscles | | Rabbit Corpuscles | | Horse Corpuscles | | Rabbit Corpuscles | |
| | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. |
| 1:6 | 80 | + | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1:12 | 30 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1:24 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1:48 | 0 | + | 0 | 0 | sp | − | 0 | 0 | 0 | 0 | 0 | 0 |
| 1:96 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1:384 | 0 | 0 | 0 | 0 | .. | .. | .. | .. | .. | .. | .. | .. |
| 1:1,536 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 1:16,144 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |

Horse corpuscle control=0. Rabbit corpuscle control=0.

The animal tested here, while showing a marked immunity to horse corpuscles, does not show immunity toward rabbit corpuscles much higher than the normal. Indeed, in the 10 normal animals studied, in two cases the lysins in the neck lymph were as concentrated as here; and in five cases the lysins in the thoracic lymph were as concentrated as here. Therefore, the lysins for rabbit corpuscles are apparently little more concentrated as may be seen by comparing Table 4 with Table 1. The agglutinins are somewhat higher than normal. Apparently the immunity is not entirely specific.

Muir and Browning[32] have advanced some evidence that a com-

plement-like body plays a rôle in agglutination. They used ox corpuscles, rabbit serum immune to ox blood, and guinea-pig serum as complement. They do not make the claim that the complement and the agglutinin are identical, merely that this form of complement behaves like hemolytic complement, is thermostable, and acts only when suitable amboceptor is present. They are not sure whether or not this is the same complement concerned in hemolysis. In the course of our experiments we found some evidence which points in the opposite direction; viz., addition of complement or at least of rabbit serum, as in one experiment, inhibits the agglutination of rabbit corpuscles by the fluids of an immune dog.

TABLE 5.

THE INHIBITION OF THE AGGLUTINATION OF RABBIT CORPUSCLES BY THE BODY FLUIDS OF AN IMMUNE DOG BY THE ADDITION OF RABBIT SERUM.

(150 c.c. rabbit blood intraperitoneally, February 16. Fluids drawn February 26. 0.1 c.c. rabbit serum as complement.)

| DILUTION | SERUM | | | | NECK LYMPH | | | | THORACIC LYMPH | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No Complement | | Complement | | No Complement | | Complement | | No Complement | | Complement | |
| | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. |
| 1:6 | 100 | − | 100 | − | 100 | − | 100 | − | 100 | − | 100 | − |
| 1:12 | 100 | − | 100 | − | 10 | + | 30 | + | 100 | − | 100 | − |
| 1:24 | 80 | + | 100 | − | 0 | + | 0 | + | 40 | + | 100 | − |
| 1:48 | 10 | + | 70 | + | 0 | + | 0 | + | 0 | + | 10 | + |
| 1:96 | 0 | + | 10 | + | 0 | + | 0 | + | 0 | + | 0 | + |
| 1:384 | 0 | + | 0 | + | 0 | + | 0 | 0 | 0 | + | 0 | + |
| 1:1,536 | 0 | + | 0 | 0 | 0 | + | 0 | 0 | 0 | + | 0 | 0 |
| 1:6,144 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 |

| DILUTION | PERICARDIAL FLUID | | | | CEREBROSPINAL FLUID | | | | AQUEOUS HUMOR | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No Complement | | Complement | | No Complement | | Complement | | No Complement | | Complement | |
| | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. | Lysis | Aggl. |
| 1:6 | 20 | + | 20 | + | 0 | + | 0 | 0 | sp | − | 0 | 0 |
| 1:12 | 0 | + | 10 | + | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 |
| 1:24 | 0 | + | 0 | + | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 |
| 1:48 | sp | − | 0 | + | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 |
| 1:96 | 0 | + | 0 | 0 | .. | .. | .. | .. | .. | .. | .. | .. |
| 1:384 | sp | − | 0 | 0 | .. | .. | .. | .. | .. | .. | .. | .. |
| 1:1,536 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 1:6,144 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |

Corpuscle control=0. Complement control=0.

This experiment shows that the addition of rabbit serum as complement instead of favoring agglutination as in the case observed by

Muir and Browning actually inhibited it in every series in the experiment by at least two dilutions. In this case we have a cerebrospinal fluid and an aqueous humor which contained agglutinins for rabbit corpuscles but in these fluids the addition of rabbit serum completely inhibited the action. We would not give the impression that this occurred normally in our work, or even frequently, for in the nine cases where complement was used with body fluids of immune dogs, this phenomenon was noted in only this one experiment. It appeared only once in the fluids of normal dogs, and in that case the action was less marked.

**Conclusions.**—1. In the blood of dogs immunized with alien blood hemolysins are found in the serum, thoracic lymph, and neck lymph, and usually in the pericardial fluid. They are not found in the cerebrospinal fluid or aqueous humor. The comparative concentration is the same as in the normal animal.

2. The addition of guinea-pig serum as complement in non-hemolytic doses increases greatly the hemolytic power of the serum, neck lymph, thoracic lymph, and pericardial fluid; therefore, in the course of immunization the amboceptors are increased in the fluids, while complement is not. Cerebrospinal fluid and aqueous humor do not become hemolytic on the addition of complement; therefore, they do not contain amboceptors.

3. In the immunized dog the agglutinins are more concentrated than in the same fluids of the normal animal. The usual order of descending concentration is: serum, thoracic lymph, neck lymph, pericardial fluid; but the order may be thoracic lymph, serum, neck lymph, pericardial fluid. Cerebrospinal fluid and aqueous humor may have agglutinins present but usually do not. If agglutinins are present in these two fluids, the concentration is about equal and lower than the pericardial fluid.

4. Immunization of a dog to horse serum increases the hemolytic power of the body fluids for horse corpuscles, but little if at all for rabbit corpuscle. The hemagglutinins are increased to a slight extent.

5. Occasionally the addition of rabbit serum will inhibit the agglutination of washed rabbit corpuscles by the fluids of a dog immune to rabbit blood.

PROTEIN PRECIPITINS.

Since most investigators who have worked with precipitins are agreed as to the delicacy and specificity of the reaction, we chose them as one of the antibodies best suited for study in our work on the body fluids of normal and immune animals. In several cases these were the same animals used in the work on hemolysins and hemagglutinins.

Our method was the same as is usually employed, namely, a dilution method. Doses of the immune fluid varying between 0.2 c.c. and 0.01 c.c. were placed in a series of test tubes and made up to 2 c.c. with sterile 0.9 per cent NaCl solution. To these tubes were then added 0.15 c.c. of the same serum as used for immunization. Control experiments were made in case of each of the fluids, and of the serum, to eliminate any possibilities of a sediment from the protein solutions confusing the results. The tubes were incubated for two hours at 37° C., and then left in the ice-box 12 to 20 hours before the final reading was made.

Our results were as follows: In the fluids of three normal dogs tested with the fresh serum of the rabbit not a trace of precipitate appeared in any tube.

In experiments with the fluids of seven dogs immune to rabbit blood, three gave positive and four negative results. One dog gave a precipitate only in the first dilution of the serum (1:10); the other mixtures and the control mixtures remained perfectly clear. This animal had been immunized by repeated injections of rabbit blood intraperitoneally, receiving in all 57 c.c. between November 14 and December 5, 1908.

A dog which 10 days earlier had received an intraperitoneal injection of 150 c.c. of rabbit blood gave a positive reaction in both serum and thoracic lymph. In this case the precipitation occurred in a much higher dilution than in the former, tho in the latter, neither the neck lymph, pericardial fluid, cerebrospinal fluid, nor the aqueous humor nor any of the controls showed any precipitate.

A dog immunized by the intraperitoneal injection of 80 c.c. of rabbit blood gave the best results of all, the serum and thoracic lymph giving precipitation in dilutions of 1:40 and the neck lymph

in 1:20; the pericardial and cerebrospinal fluids and the aqueous humor, however, gave no reaction in 1:10.

Of the five attempts to produce precipitins in dogs by immunization with horse serum, four were entirely negative, and even in the one positive result only the serum, thoracic lymph, and the neck lymph contained precipitin. The precipitation occurred in a higher dilution in the thoracic lymph than in the serum, but only in the lower dilutions of the neck lymph, which was considerably weaker than the serum.

Our data are not uniform enough nor extensive enough to warrant us in drawing conclusions. It is apparent that dogs develop precipitins with extreme difficulty, and that a good method of immunization is by single, large, intraperitoneal injections. The results that we have seem to show that the precipitins follow closely the hemagglutinins and the hemolysins in their distribution in the various body fluids of immune animals, altho we have not as yet been able to demonstrate any in the pericardial fluid.

### BACTERIAL AGGLUTININS.

We also made a study of the concentration of the agglutinins for the typhoid bacillus in the various body fluids of normal and immunized cats and dogs, and intend later to extend the work to cover the bacteriolysins.

Nuttall,[34] employing both the hanging drop method and the plate method, found bacteriolysins for the anthrax bacillus in the aqueous humor and the pleuritic exudate of dogs and rabbits. Prudden[38] found bacteriolysins in the amniotic, hydrocele, and acites fluids. Meltzer and Norris[28] found the thoracic lymph nearly as bacteriolytic as the serum for the typhoid bacillus. Widal[43] found agglutinins for the typhoid bacillus active in a dilution of 1:60 in the pericardial fluid, while the serum of the same patient was active at 1:350. Edema fluid was found strongly agglutinating also, but the result with cerebrospinal fluid was negative. Pick[37] found that cerebrospinal fluid agglutinated at a dilution of 1:1 and 1:2. Kohler[20] found agglutinins in the cerebrospinal fluid in one case in ten examined of non-typhoid patients, and but three times out of 19 cases of typhoid fever, and these in dilutions of 1:1, 1:5, and 1:10. Braude and Carlson[7] made a study of the concentration of the agglutinins for the typhoid bacillus in the body fluids of normal and immunized cats and dogs. Inasmuch as they used for comparison the action of the fluids of the same animal upon the same bacterial suspension, their results give more nearly the true conditions of the body fluids in an animal; they avoided the error which a comparison of the body fluids of different animals is sure to introduce on account of the wide individual variations. The hanging drop method which they employed, however, is not considered as accurate as the pre-

cipitation method which we used. They found bacterial precipitins in the cerebrospinal fluid of immunized dogs and cats.

The fluids used in these experiments were collected as described at the beginning of this paper. The test tubes were the same as those used in the hemolytic work. The bacteria were secured from 20–24-hour slant agar cultures, made up with sterile 0.9 per cent NaCl solution to a distinctly cloudy suspension, and then filtered through filter paper to remove all of the clumps. A series of tubes was arranged for each fluid, then after the proper amount of fluid had been measured into each, the bacterial suspension was added. The dilutions used were 1:10, 1:50, 1:100, 1:500, 1:2,000, 1:6,000. Our lowest dilution was, perhaps, too high to detect the traces of agglutinins reported by Pick and Kohler. The tubes were all incubated together at 37° C. for two hours and then kept in the ice-box for from 12–20 hours before the final observations.

In our study of agglutinins for the typhoid bacillus we used normal cats and dogs, animals actively immunized by the repeated injection of typhoid cultures, and animals rendered passively immune by the withdrawal of large quantities of blood from the normal animal, and the subsequent injection of an equal amount of warm, defibrinated blood from an actively immunized animal of the same kind.

**A. Normal animals.**—We studied first the concentration of agglutinins in the body fluids of normal cats and found agglutinins for the typhoid bacillus active in a dilution from 1:10 in the serum and the thoracic lymph; the neck lymph and the pericardial fluid usually contain them in the same concentration but the cerebrospinal fluid and the aqueous humor do not contain them in such dilution.

DOGS.—A study of the concentration of the typhoid agglutinins in normal dogs gave the results shown in the following table:

TABLE 6.

THE COMPARATIVE AGGLUTINATING POWER ON THE TYPHOID BACILLUS OF THE BODY FLUIDS OF A NORMAL DOG.

| Dilution | Serum | Neck Lymph | Thoracic Lymph | Pericardial Fluid | Cerebro-spinal Fluid | Aqueous Humor |
|---|---|---|---|---|---|---|
| 1:10 | ++ | ++ | ++ | o | o | o |
| 1:50 | ++ | o | o | o | o | o |
| 1:100 | + | o | o | o | o | o |
| 1:500 | o | o | o | o | o | o |

This experiment is one of four performed on normal animals, and gives a fair idea of the concentration of the agglutinins in the body fluids. There are, of course, variations between animals even in the same species. In all of our experiments the concentration of agglutinins was highest in the serum, less in the thoracic and the neck lymph, and the pericardial fluid contained agglutinins in only one case. The cerebrospinal fluid and the aqueous humor did not contain any in any case.

**B. Actively immunized animals.**—We made a study of the fluids of five immunized cats. Three had received repeated subcutaneous injections of typhoid bacilli. For the first few injections killed cultures, but later live cultures were used. The doses injected were increased gradually and careful record of the weight and general condition kept, to guard against pushing the process too rapidly. The remaining two animals received a single large injection of six living 24-hour cultures. The results given in Table 7 may be taken as fairly typical.

TABLE 7.

THE COMPARATIVE AGGLUTINATING POWER ON THE TYPHOID BACILLUS OF THE BODY FLUIDS OF AN IMMUNIZED CAT.

(Immunized by repeated subcutaneous injections. Fluids collected five days after the last injection.)

| Dilution | Serum | Neck Lymph | Thoracic Lymph | Pericardial Fluid | Cerebro-spinal Fluid | Aqueous Humor |
|---|---|---|---|---|---|---|
| 1:10 | ++ | ++ | ++ | + | ? | ? |
| 1:50 | ++ | ++ | ++ | o | o | o |
| 1:100 | ++ | ++ | ++ | o | o | o |
| 1:500 | ++ | o | + | o | o | o |
| 1:2,000 | + | o | o | o | o | o |
| 1:6,000 | + | o | o | o | o | o |

Control = o.

The other experiments showed some variations. In two cases in five the agglutinins were as concentrated in the thoracic lymph as in the serum, in the remaining three of five the serum was by far the more concentrated of the two. In one case the neck lymph showed the same concentration of agglutinins as the thoracic lymph, but both were considerably lower than the serum; in the four remaining cases the concentration in the neck lymph was lower than in the thoracic lymph. Agglutinins were found in four of five cases in the pericardial fluid, but in no case in a dilution higher than 1:10. Agglutination in the fifth case was questioned. The cerebrospinal fluid

was negative in all dilutions used in four of five cases, and agglutination in the fifth case at 1:10 was questioned. The aqueous humor gave negative results in three of five cases, and the agglutination was questioned in the remaining two at a dilution of 1:10.

The development of agglutinins for the typhoid bacillus in dogs runs a course which is strictly comparable to that in the cats under similar circumstances.

TABLE 8.

THE COMPARATIVE AGGLUTINATING POWER FOR THE TYPHOID BACILLUS OF THE BODY FLUIDS OF AN IMMUNE DOG.

(Immunized by repeated subcutaneous injections.)

| Dilution | Serum | Neck Lymph | Thoracic Lymph | Pericardial Fluid | Cerebrospinal Fluid | Aqueous Humor | Control |
|---|---|---|---|---|---|---|---|
| 1:10 | ++ | ++ | ++ | ++ | o | o | o |
| 1:50 | ++ | ++ | ++ | ++ | o | o | .. |
| 1:100 | ++ | ++ | ++ | + | o | o | .. |
| 1:500 | ++ | + | ++ | o | .. | .. | .. |
| 1:2,000 | + | o | + | o | .. | .. | .. |
| 1:6,000 | o | o | o | o | .. | .. | .. |

From Table 8 it is evident that the concentration of the agglutinins in the body fluids of immune dogs runs a course parallel to that in the immune cats. The concentration in the neck lymph and the pericardial fluid is considerably lower than that in the thoracic lymph and serum, and of the two the concentration in the neck lymph is the higher. In our seven experiments on typhoid immune dogs, thoracic lymph and serum showed the same concentration of bacterial agglutinins in four cases; in the remaining three cases the concentration is greater in the serum. The pericardial fluid contained agglutinins in six of seven cases. The highest dilution at which agglutination occurred was 1:100 (three cases). In no case were agglutinins found in the cerebrospinal fluid in the dilutions used. Traces of agglutinins were observed in three of six cases in the aqueous humor in a dilution of 1:10.

We considered it of interest to determine whether immunity to rabbit blood affected in any way the agglutinins for the typhoid bacillus in the body fluids of a dog. With this in mind we tested the usual six fluids of one of our immune dogs. An increased agglutinating power could be expected, if, in terms of Ehrlich's hypothesis, there were any cell receptors common both to blood cells and the typhoid bacilli.

TABLE 9.

THE COMPARATIVE AGGLUTINATING POWER ON THE TYPHOID BACILLUS OF THE BODY FLUIDS OF A DOG IMMUNIZED WITH RABBIT BLOOD.

(Intraperitoneal injections as follows: October 10, 10 c.c.; October 19, 8 c.c.; October 24, 8 c.c.; October 29, 9 c.c.; November 3, 10 c.c.; November 9, 10 c.c.; November 14, 10 c.c.; fluids drawn November 24.)

| Dilution | Serum | Neck Lymph | Thoracic Lymph | Pericardial Fluid | Cerebro-spinal Fluid | Aqueous Humor |
|---|---|---|---|---|---|---|
| 1:10 | ++ | + | + | o | o | o |
| 1:50 | + | o | + | o | o | o |
| 1:100 | + | o | o | o | o | o |
| 1:500 | o | o | o | o | o | o |

A comparison of this table with Table 7 shows that the agglutinins for the typhoid bacillus were little if any higher than those of the normal animal, altho this was one of our most highly immunized animals and agglutinated rabbit corpuscles strongly in a dilution of 1:1,536 in the serum and in a dilution of 1:384 in the neck and the thoracic lymph. The relative concentration in the body fluids is strictly comparable to those in the normal animal cited. We tested also the blood serum of two other dogs immune to rabbit blood with exactly similar results.

**C. Passive immunity.**—Evidence that antibodies of various kinds are able to pass through membranes is not lacking in the literature. Ehrlich[10] found that the young from a mouse immune to abrin, ricin, or robin possess an immunity to these poisons which persists for two months. Ascoli[1] found that the antibodies of the new born child come from the maternal circulation and are not formed in the fetus itself. This being true, the antibodies of the fetus have penetrated the walls of at least a double membrane. Merkle[29] found that the same was true for rabbits, since the young born of a mother immune to human blood contain antibodies for human blood, altho they suckle a normal mother from the very first. Lüdke[23] confirmed the work of Merkle. Ricketts[39] has shown that the young born to a guinea-pig mother immune to Rocky Mountain spotted fever possess an immunity to that disease, altho they suckled a normal mother. DeBlasi[8] has shown that if a cat is immunized *post partum* to B. dysenteriae the young develop an immunity from the milk. This is even a more striking example than the others of the ability of antibodies to penetrate membranes, for in this case the antibodies after reaching the alimentary canal of the young animal must penetrate

the intestinal mucosa, and later the capillary or lymphatic wall, depending upon whether the path of absorption was the blood or the lymph.

It was hoped that a careful study of the passage of these antibodies from the blood into the various body fluids in a passively immune animal would throw some light upon the problem of lymph formation. We have been disappointed thus far, for our results have not been decisive enough to warrant any general conclusions.

Passive immunity was established by the withdrawal under light ether anesthesia in most cases of a large amount of blood from a normal animal, and the subsequent injection of an equal amount of warm, defibrinated blood from an actively immune animal of the same kind.

CATS.—To show the concentration of agglutinins in the body fluids of a passively immune cat we give Table 10 as typical of our results. It will also show the concentration of agglutinins of the actively immune cat from which the blood was taken.

TABLE 10.

THE COMPARATIVE AGGLUTINATING POWER ON THE TYPHOID BACILLUS OF THE BODY FLUIDS OF CATS ACTIVELY AND PASSIVELY IMMUNE TO THE TYPHOID BACILLUS.

(Cat 11, immunized June 29 by the injection subcutaneously of six live 24-hour slant agar cultures of the typhoid bacillus, operated July 9. Cat 12, passively immunized by the withdrawal on July 9 of 100 c.c. of blood and the injection of 100 c.c. of blood from Cat 11. Operated July 10.)

| DILUTION | SERUM | | | NECK LYMPH | | THORACIC LYMPH | | PERICARDIAL FLUID | | CEREBROSPINAL FLUID | | AQUEOUS HUMOR | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 11 | 12* | 12 | 11 | 12 | 11 | 12 | 11 | 12 | 11 | 12 | 11 | 12 |
| 1:10..... | ++ | + | ++ | ++ | ++ | ++ | ++ | + | o | o | o | o | o |
| 1:50..... | ++ | + | + | ++ | + | ++ | + | + | o | o | o | o | o |
| 1:100..... | ++ | o | + | + | o | + | + | o | o | o | o | o | o |
| 1:500..... | + | o | + | + | o | + | o | o | o | o | o | o | o |
| 1:2,000..... | + | o | o | + | o | + | o | o | o | o | o | o | o |
| 1:6,000..... | o | o | o | o | o | o | o | o | o | o | o | o | o |

* Normal serum before injection of immune serum 11.

From Table 10 it is evident that it is possible to increase the agglutinins not only in the serum but in both of the lymphs by a process of passive immunization. The relative concentration is maintained in the fluids which we find in the actively immunized animals of the same grade of immunity. In no case in the passively immunized animal did the pericardial fluid show any increase over the normal animal. The cerebrospinal fluid and the aqueous humor showed no

increase, and, indeed, one would not expect that they would, since in the actively immune animal the presence of antibodies is the exception and not the rule. The results here are typical of all of our results. In four passively immune cats this same increase in the concentration of the agglutinins in serum, neck lymph, and the thoracic lymph was noted. In two of the four cases the concentration in the neck lymph and the thoracic lymph was equal, in the remaining two of the four experiments the concentration in the thoracic lymph was greater than that in the neck lymph. There appears on the whole to be a tendency for the concentration of the agglutinins in the thoracic and the neck lymph in the passively immune animals to run more nearly parallel than in the actively immunized animals.

DOGS.—Passive immunity produced in dogs in the manner described yields exactly similar results; namely, the concentration in agglutinins of the serum, neck lymph, and thoracic lymph can be greatly increased. The concentration of the agglutinins in the pericardial fluid, cerebrospinal fluid, and the aqueous humor is no higher than in the normal animal.

In one of three passively immunized dogs the concentration in the neck lymph and thoracic lymph was equal; in the other two the thoracic lymph was much higher. The fact that 50 per cent of our passively immunized cats and 33 per cent of the passively immune dogs showed an equal concentration of the agglutinins in the thoracic and the neck lymph may be significant, for the percentage of such findings in the actively immune animals is 14 per cent for the dogs and 16 per cent for the cats; but much more extensive experimentation is necessary before any reliance can be placed on these results.

In order to determine the relative rapidity with which these antibodies pass from the blood into the lymph the following experiment was made. A dog was anesthetized and rendered passively immune by the withdrawal of 300 c.c. of blood from the femoral artery and the injection of 300 c.c. of blood and serum from a typhoid immune dog. Small samples of blood were drawn at stated intervals, as were also samples of neck lymph. Thoracic lymph, pericardial fluid, cerebrospinal fluid, and aqueous humor were collected at the end of the experiment. All of the fluids except the thoracic lymph

which was tinged with blood were in good condition and were tested in the usual manner. The results are shown in Table 11.

TABLE 11.

THE RATE OF PASSAGE OF ANTIBODIES FROM THE BLOOD INTO THE BODY FLUIDS IN PASSIVELY IMMUNE ANIMALS.

| Fluid | Highest Dilution Showing Agglutination |
|---|---|
| Normal serum | 1:100 |
| Immune serum injected into passively immune dog | 1:2,000 |
| Equal parts of normal and immune sera | 1:2,000 |
| Serum 25 min. after transfusion | 1:500 |
| " 1 hr. 25 min. after transfusion | 1:500 |
| " 2 " " " " " | 1:500 |
| " 3 " " " " " | 1:500 |
| " 4 " " " " " | 1:500 |
| Neck lymph 3 hr. " " | 1:50 |
| " " 4 " 30 min. after transfusion | 1:50 |
| Thoracic lymph 4 hr. 30 min. after transfusion | 1:100 |
| Pericardial fluid 4 " 30 " " " | 0 |
| Cerebrospinal fluid | 0 |
| Aqueous humor | 0 |

It may be noted that the results of this experiment are practically identical with those secured from our 24-hour experiments on the passively immunized dogs. The concentration of the agglutinins of the serum remain practically the same during the $4\frac{1}{2}$ hours of this experiment. The neck lymph had the same concentration of agglutinins three hours after the transfusion that it had at the close of the experiment. The thoracic lymph in this case showed considerably higher than the neck lymph, part of which may have been due to the serum in the lymph, which came either through the increased permeability of the capillary walls due to the action of the anesthetic or to trauma, or to the natural anastomoses between the lymphatics and the blood vessels of the splanchnic area.

The above experiment seems to indicate that the passage of such substances as bacterial agglutinins from the blood to the lymphs is a relatively rapid process, for the concentration of these bodies was the same in the body fluids in $4\frac{1}{2}$ hours as in 24 hours after passive immunization.

From the results obtained the following conclusions seem justified:

1. Agglutinins for the typhoid bacillus are found in the serum and thoracic lymph of normal cats in approximately equal amounts.

They may or may not be present in the neck lymph and the pericardial fluid. They are not found in the cerebrospinal fluid nor the aqueous humor.

2. Agglutinins for the typhoid bacillus are found in the serum, neck lymph, and the thoracic lymph in normal dogs in relatively higher concentrations than in the same fluids in normal cats. They are most concentrated in the serum, are in nearly equal concentration in the two lymphs. They may or may not be found in the pericardial fluid. They are found neither in the cerebrospinal fluid nor the aqueous humor.

3. Agglutinins for typhoid bacilli are found in actively immune cats in the serum, thoracic lymph, neck lymph, and pericardial fluid in decreasing concentration in the order mentioned. If found in the cerebrospinal fluid and aqueous humor there are only traces.

4. Agglutinins for typhoid bacilli are found in actively immune dogs in the serum, thoracic lymph, neck lymph, and pericardial fluid, usually in decreasing concentration in the order named. Serum and thoracic lymph may show an equal concentration. Cerebrospinal fluid shows no agglutinins in a dilution of 1:10. Aqueous humor may or may not show agglutinins in a dilution of 1:10.

5. Immunization of a dog to rabbit blood does not increase the concentration of agglutinins for typhoid bacilli in the body fluids above the normal.

6. In the passively immunized animal the agglutinins for typhoid bacilli pass readily from the blood stream into the lymphs, usually in greater concentration into the thoracic lymph than into the neck lymph, but the concentration may be equal in the two, showing the permeability of the two systems to be the same. They do not pass into the pericardial fluid, cerebrospinal fluid, nor the aqueous humor.

7. The passage of the agglutinins from the serum to the lymphs in the passively immunized animal is a relatively rapid process, and no difference is shown between the concentration at the end of $4\frac{1}{2}$ hours and 24 hours.

## OPSONINS.

We made a quantitative study of the bacterial opsonins and the hemopsonins in the body fluids of normal and immunized dogs.

Since the discovery of the opsonins by Wright and Douglas[44] there has been a great deal of work done with these bodies, and as a result there has arisen an extensive and conflicting literature. It is beyond the scope of this paper to undertake a general discussion of this subject. We will confine ourselves, therefore, to those results which most nearly relate to our problem. So far as we have been able to determine, there has been up to this time no study of the relative concentration of the opsonins in the different body fluids of the same animal.

Wright and Reid[45] found that exudates may contain little or no opsonin. Opie[35] found that the exudates following the injection of bacteria or turpentine were practically opsonin free if unmixt with blood. Böhme[6] reports 15 cases of pleural and peritoneal exudates showing a concentration of the opsonins ranging from less than half to even a greater concentration than that of the serum. He also examined the edema fluid in seven persons. In two of these cases there was very little opsonin while the remaining five showed a considerable amount, but in no case was it equal to that of the serum. This same investigator examined the cerebrospinal fluid in 16 cases and obtained opsonic indices varying from 8 per cent to 76 per cent. He notes that these fluids were free from blood. Böhme tries to correlate the concentration of the opsonin in these cases with the amount of the protein in the fluid.

Levaditi and Inman[21] and others have shown that while the aqueous humor normally contains little or no opsonin, yet that secured after a previous withdrawal contains the opsonin.

**Methods.**—The method of securing the fluids was the same as that described in the foregoing.

The leukocytes were from the pleural exudate of young, healthy dogs which 24 hours previously had been given intraplural injection of a suspension of aleuronaut in sterile 0.9 per cent NaCl solution. The exudate was secured after bleeding the animal to death, and was drawn into a warm solution of 1 per cent sodium citrate in 0.9 per cent NaCl. The leukocytes were centrifugated out, and washed a second time in warm, sterile salt solution.

The bacterial emulsion was obtained by suspending in 0.9 per cent NaCl solution 24-hour slant agar cultures of Staph. aureus. This emulsion was filtered through absorbent cotton in order to remove the clumps, and was then made up to the desired opalescence. In all of the experiments a fairly rich suspension of the bacteria was used, but no attempt was made to standardize this emulsion, and comparison of individual experiments, except where the same suspension of leukocytes and bacteria were used, would not be warranted.

The technic was essentially that described by Walker with slight modification. This method in our hands gave the most satisfactory results. Dilutions of the various body fluids were used in the same way as the whole fluid. The usual time for incubation was 20 minutes. Care was taken to make the smear from the incubated mixture as soon after the period of incubation as possible, so that the time factor was as nearly equal as it could be kept. We here incur a slight source of error in the difference in the age of the material, for Beattie[5] has shown that the older citrated leukocytes are up to 24 hours, the greater the phagocytosis, but the maximum difference in time between the first and last smear was never greater than one hour. Great care was taken to keep the leukocytic suspension of the same consistency throughout the experiment. The leukocytes were used as fresh as possible, usually in about two hours or less after removal from the animal's body. The number of leukocytes varied from

60 to 100 for each dilution, but the number was kept constant for each experiment. The stains used were carbol-thionin and Giemsa's blood stain, but the same stain was used throughout the same series of fluids.

Inasmuch as there has been so much criticism of the opsonic methods we made the following tests of the accuracy of our observations. Using the same fluids, leukocytes, and bacterial suspension, duplicate tests were made. The slides were labeled in such a way that the person making the count had no way of knowing what the slide contained, thus eliminating the personal equation. The result of this test was that while most of the duplicates agreed fairly closely there were cases that varied as much as 25 per cent, but the majority of the counts showed a much closer agreement.

**Bacteriopsonins.**—We give the results of an experiment which we consider as typical so far as concerns opsonin for Staph. aureus in normal dogs.

TABLE 12.

COMPARATIVE STUDY OF THE OPSONIN FOR STAPH. AUREUS IN THE BODY FLUIDS OF A NORMAL DOG

| Dilution | Serum | Neck Lymph | Thoracic Lymph | Pericardial Fluid | Cerebro-spinal Fluid | Aqueous Humor |
|---|---|---|---|---|---|---|
| Whole..... | 3.40 | 3.50 | 4.17 | 0.81 | 1.01 | 1.08 |
| 1:10..... | 0.30 | 0.16 | 0.58 | 0.32 | .... | .... |

Control=0.36.

It will be noted that in this experiment the opsonin had practically disappeared in the dilution of 1:10 in all of the fluids. In the undiluted fluids the concentration of the opsonin in the serum, neck, and thoracic lymph was nearly the same, but there was a slight excess in favor of the thoracic lymph. The pericardial fluid, cerebrospinal fluid, and the aqueous humor contained opsonin in a very much lower concentration.

In four of nine normal animals the concentration of the opsonin in the serum was considerably higher than in the thoracic and the neck lymph. In three of nine cases the concentration was practically equal in the serum and the two lymphs. In five of eight experiments the amount of opsonin in the neck lymph was considerably less than in the serum and thoracic lymph. It is thus evident, that, as far as these three fluids are concerned, the concentration is greatest, on the average, in the serum, least in the neck lymph, while the thoracic lymph occupies an intermediate position.

Opsonin was found in the cerebrospinal fluid of four of seven dogs, but in every case it was in decidedly smaller amounts than in the serum and lymphs of the same animal. In the other three animals there was no opsonin in this fluid.

The aqueous humor contained opsonin in five of eight cases, but in only one of these did the concentration approach that of the serum of the same animal. The remaining four showed very much less phagocytosis, while three experiments gave negative results.

Opsonin was found in the pericardial fluid in two of seven dogs in considerable amounts, in one it was practically equal to the neck lymph of the same animal. In one of the remaining animals there was a trace of opsonin. The other four animals contained no opsonin in this fluid.

It is difficult to increase to any marked extent the concentration of the opsonin for Staph. aureus in a dog by immunization.

We submit below two experiments in detail which we consider quite typical of our results.

TABLE 13.

COMPARATIVE OPSONIC POWER FOR STAPH. AUREUS OF THE BODY FLUIDS OF IMMUNIZED DOGS.

(Both of these dogs received subcutaneously six slant agar cultures of Staph. aureus suspended in 0.9 per cent NaCl solution. Ten days later the fluids were all secured in apparently perfect condition. Fluids kept over night in ice-box. Bacteria from a 24-hour slant agar growth. Incubation 20 minutes. Carbol-thionin. Same bacterial suspension and leukocytes in both experiments.)

| | Dilution | Serum | Neck Lymph | Thoracic Lymph | Pericardial Fluid | Cerebrospinal Fluid | Aqueous Humor |
|---|---|---|---|---|---|---|---|
| Dog I..... | Whole | 9.75 | 4.51 | 5.95 | 3.30 | 1.85 | 0.78 |
| | 1:10 | 4.51 | 1.75 | 3.51 | 1.33 | 0.88 | 0.50 |
| | 1:50 | 1.75 | 0.86 | 1.01 | 0.22 | 0.28 | 0.28 |
| Dog II..... | Whole | 10.07 | 6.67 | 8.13 | 4.18 | 0.92 | 1.33 |
| | 1:10 | 4.21 | 1.50 | 2.88 | 1.47 | 0.22 | 0.30 |
| | 1:50 | 0.90 | 0.38 | 0.52 | 0.17 | 0.22 | 0.35 |

Control for both=0.22.

It will be noted that the results of these experiments are comparable with those with respect to the other antibodies studied. In the two experiments cited above the concentration of the opsonin is considerably higher in the serum than in the other fluids. Of the two lymphs there is a slight excess in that from the thoracic duct over that from the cervical lymphatics. In both of these experiments while there is considerable opsonin in the pericardial fluid yet it is less than in the serum and the lymphs. In both of these experiments the cerebrospinal fluid and the aqueous humor contained opsonin, but in very much smaller amounts than in the corresponding serum and lymphs.

**Conclusions.**—There is a considerable amount of opsonin for

Staph. aureus in the body fluids of normal dogs. The amount found in immune dogs is quite comparable with this, as regards both the amount and the relative distribution.

Opsonin may be present in considerable concentration in all of the body fluids studied by us, but in the serum, thoracic, and neck lymphs they are always found in greater concentration than in the other fluids studied. Of these fluids the serum usually contains the greatest amount, and the thoracic lymph usually more than the corresponding neck lymph. The pericardial fluid, cerebrospinal fluid, and the aqueous humor may or may not contain opsonin, but rarely in amounts comparable to those of the serum and lymphs.

## HEMOPSONINS.

It has long been noted that red corpuscles were taken up by phagocytes under certain conditions but it was not until opsonins were discovered by Wright and Douglas that the rôle of the serum in phagocytosis was understood. Neufeld and Töpfer[33] showed that there was something termed by them "hemotropic substance" in the serum of a rabbit immune to goat blood, which caused the phagocytosis of goat erythrocytes by guinea-pig leukocytes *in vitro*. Barrat[3] found the same antibody in the serum of doves immune to hen blood. He proved these bodies thermostable. Hektoen [6] was the first to point out the similarity of these substances to bacterial opsonins and suggested the name "hemopsonins." Neufeld and Töpfer, Barrat, Keith, and Hektoen all cite evidence to show that these substances are distinct from the amboceptors of the hemolysins. The most extensive recent work on the hemopsonins is by Hektoen. [7] He shows that normal serum may contain hemopsonins for heterologous or even homologous erythrocytes; that immune hemopsonins are highly resistant to heat; and are in part specific and in part non-specific.

**Methods.**—The body fluids of the dogs were secured as described earlier in this paper, and inactivated by heat at 55° C. for 30 minutes. Rat corpuscles were used throughout the work. They were made up to 5 per cent suspension after careful washing in 0.9 per cent NaCl solution. The technic followed was to measure into each of a series of small test tubes quantities of fluids ranging between 0.2 c.c. and 0.002 c.c. and then adding enough salt solution to make 0.2 c.c. To this was then added 0.4 c.c. of a mixture of equal quantities of rat corpuscles and leukocytic suspension. In this way dilutions varying between 1:3 and 1:300 were secured. The tubes were incubated in a shaker for one hour. Then the contents were mixt thoroughly, smears made, and fixt in absolute alcohol for one hour, and then stained with Giemsa's stain for 30–60 minutes. The percentage was figured from the number of the leukocytes actively phagocytic. No attempt was made to count the number of erythrocytes engulfed per leukocyte. In every case 500 leukocytes were counted. Our figures will represent, then, the percentage of leukocytes phagocytic, and shows only the activity of thermostable hemopsonins.

Early in the work an experiment was performed to test the accuracy of the method. Two complete sets of tests were made with the fluids of a normal dog, smears were

made, fixt, and stained in the ordinary way. Labels were then attached to the slides and one of us numbered the slides in a haphazard manner and kept a record of the numbering used. The slides were all mixt together and then counted by the other. In this way the subjective factor would not enter at all. The results were highly satisfactory, for by counting 500 leukocytes and estimating the percentage from that number, the corresponding slides agreed almost exactly. In one case there was a difference amounting to over 2 per cent.

NORMAL DOGS.—The first point was to determine the concentration of the hemopsonins in the body fluids of normal animals. As a typical experiment we cite Table 14.

TABLE 14.

COMPARATIVE HEMOPSONIC POWER FOR RAT CORPUSCLES OF THE BODY FLUIDS OF A NORMAL DOG.

| Dilution | Serum | Neck Lymph | Thoracic Lymph | Pericardial Fluid | Cerebro-spinal Fluid | Aqueous Humor |
|---|---|---|---|---|---|---|
| 1:3 | 3.20 | 1.20 | 0.60 | 0.00 | 0.40 | 0.20 |
| 1:6 | 0.20 | 0.00 | 0.20 | 0.00 | 0.00 | 0.00 |
| 1:12 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Control = 0.

This table shows that the concentration of hemopsonins in the various body fluids runs practically parallel with that of the other antibodies. It is highest in the serum. The two lymphs are lower but practically equal. There are also traces in the other fluids in this case. In another normal animal the same conditions were found.

ACTIVELY IMMUNIZED DOGS.—In establishing immunity for the work on hemopsonins we followed a uniform technic. Each animal received intravenously 0.5 c.c. of a 5 per cent solution of washed rat corpuscles per kilo of body weight. The animal was operated the 10th day after the injection. We cite the experiment in Table 15 as typical.

TABLE 15.

COMPARATIVE HEMOPSONIC POWER FOR RAT CORPUSCLES OF THE BODY FLUIDS OF AN IMMUNE DOG.

| Dilution | Serum (Normal) | Serum | Neck Lymph | Thoracic Lymph | Pericar-dial Fluid | Cerebro-spinal Fluid | Aqueous Humor |
|---|---|---|---|---|---|---|---|
| 1:3 | 24.4 | 45.0 | 43.4 | 39.4 | 25.8 | 10.8 | 4.8 |
| 1:6 | 4.2 | 38.4 | 28.0 | 28.2 | 15.0 | 2.6 | 1.0 |
| 1:12 | 1.8 | 24.6 | 5.0 | 14.6 | 7.0 | 1.2 | 0.0 |
| 1:24 | 0.4 | 5.8 | 3.0 | 2.2 | 2.0 | 0.2 | 0.0 |
| 1:60 | 0.0 | 2.6 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 1:120 | ... | 1.0 | 0.8 | 0.0 | 0.0 | 0.0 | 0.0 |
| 1:300 | ... | 0.2 | 0.0 | 0.0 | ... | ... | ... |

Table 15 shows that a considerable degree of immunity was reached, the immune serum producing a much higher degree of

phagocytosis than the normal. The concentration was highest in the serum; the two lymphs are practically parallel with a slight balance in favor of the neck lymph, because it not only produces a higher percentage of phagocytosis in the lowest dilution, but also causes phagocytosis in a much higher dilution. The pericardial fluid shows a somewhat lower concentration than the lymphs; the cerebrospinal fluid and the aqueous humor contain the opsonin in lower concentration than the pericardial fluid.

In six experiments with immune dogs the concentration of hemopsonins ran the course shown in this experiment. We find some variations in the cerebrospinal fluid and the aqueous humor. In three out of five cases where the cerebrospinal fluid and aqueous humor were tested the concentration of the hemopsonins was higher in the former than in the latter. The fourth case is the one cited in Table 15, and in the fifth case neither showed hemopsonins. In one of six cases the hemopsonins of the pericardial fluid and aqueous humor were practically equal in concentration.

PASSIVELY IMMUNE DOGS.—Passive immunity was produced in the same way described under bacterial agglutinins. We cite here as an example the results obtained in the case of a dog immunized by injecting intravenously 300 c.c. of the blood of an immune dog after first removing 260 c.c. by bleeding.

TABLE 16.

COMPARATIVE HEMOPSONIC POWER FOR RAT CORPUSCLES OF THE BODY FLUIDS OF A DOG PASSIVELY IMMUNIZED TO RAT BLOOD.

| Dilution | Serum (Normal) | Serum | Neck Lymph | Thoracic Lymph | Pericardial Fluid | Cerebrospinal Fluid | Aqueous Humor |
|---|---|---|---|---|---|---|---|
| 1:3 | 26.0 | 36.4 | 15.6 | 32.0 | 3.6 | 0.8 | 0.4 |
| 1:6 | 16.0 | 27.2 | 7.8 | 12.0 | 1.0 | 0.0 | 0.2 |
| 1:12 | 4.6 | 13.6 | 1.6 | 2.6 | 0.0 | 0.0 | 0.0 |
| 1:24 | 0.4 | 2.6 | 0.0 | 1.6 | 0.0 | ... | ... |
| 1:60 | 0.0 | 1.8 | 0.0 | 0.4 | ... | ... | ... |
| 1:120 | ... | 0.6 | ... | 0.0 | ... | ... | ... |
| 1:300 | ... | ... | ... | ... | ... | ... | ... |

The results in our experiment in passive immunity show first, an increase in the concentration of the hemopsonins in the serum, and second, a marked increase in the concentration of the antibodies in the thoracic lymph, bringing it up to a concentration equal to that of the serum, while the concentration of the hemopsonins in the neck

lymph remains low and from a comparison with other animals we would say that the injection of the highly immune serum had had no effect upon the concentration of the hemopsonins in the neck lymph. The same is true of the pericardial fluid, the cerebrospinal fluid, and the aqueous humor, where the concentration is no higher than in the normal dog. It must be remembered that this dog showed a relatively high hemopsonic power before immunization by injection of the immune blood. We have other experiments which show the same phenomenon.

From these experiments we draw the following conclusions:

1. Hemopsonins are found in the body fluids of normal dogs. The concentration is highest in the serum, lower in the thoracic and neck lymph, which run almost parallel, and are found in the other body fluids only in traces.

2. By a process of immunization the hemopsonins of the body fluids can all be increased. The order of descending concentration is the serum, neck lymph, thoracic lymph, pericardial fluid, aqueous humor, and the cerebrospinal fluid. Sometimes the arrangement in the scale of the last two is reversed.

3. Hemopsonins can pass from the blood into the thoracic lymph, but apparently they do not pass into the lymph of the head region. The concentration of the hemopsonins in the pericardial fluid, cerebrospinal fluid, and aqueous humor is not increased in 24 hours over the normal by passive immunization as in our experiments.

## GENERAL CONCLUSIONS.

In the normal animal the concentration of hemolysins, hemagglutinins, bacterial agglutinins, bacterial opsonins, and hemopsonins decrease in the body fluids in the following order: serum, thoracic lymph, neck lymph. Traces of hemagglutinins, bacterial agglutinins, and opsonins are found in the pericardial fluid. Traces of opsonins are found in the cerebrospinal fluid and aqueous humor. No precipitins for rabbit serum were found in any of the body fluids.

Immunization increases the concentration of the antibodies named above in the fluids in which they are found in the normal animal. Hemolysins are sometimes found in the pericardial fluid, and in a few cases traces of bacterial agglutinins in the cerebrospinal fluid and

aqueous humor. Protein precipitins are found in the serum, thoracic lymph, and neck lymph, the concentration being lower in the last than in the first two.

The immunity is specific in the other body fluids as in the serum.

The introduction of an immune blood into a normal animal increases the concentration of the bacterial agglutinins in both the lymphs above the normal. The introduction of an immune blood does not increase the concentration of hemopsonins in the neck lymph, altho the concentration in the thoracic lymph is markedly increased. The concentration of bacterial agglutinins and hemopsonins in the cerebrospinal fluid is not modified by passive immunization.

The passage of antibodies from blood to the lymph is a relatively rapid process.

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